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Original Investigations

The Toxicological Properties of Impurities in Malathion

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Abstract. During a malaria eradication programme in Pakistan in 1976, out of 7,500 spraymen, 2,800 became poisoned and 5 died. The major determinant of this poisoning has been identified as isomalathion present as an impurity in the malathion. It seems almost certain that the isomalathion was produced during storage of the formulated malathion.

The quantitative correlation found between isomalathion content and toxicity of many field samples of malathion has been confirmed by an examination of mixtures of pure compounds. Addition of known amounts of isomalathion to technical malathion indicates that other active substances are present. These impurities have been identified (trimethyl phosphorothioates) and have been shown to behave like isomalathion in potentiating the toxicity of malathion. Some preliminary work on their toxicological properties is reported.

The mechanisms involved in the potentiation of the toxicity of malathion are discussed.

Key words: Malathion – Isomalathion – Trimethyl phosphorothioates – Acetylcholinesterase – Carboxylesterase – Potentiation of toxicity.

Introduction

During the malaria eradication programme in Pakistan in 1976, out of about 7,500 spraymen, 2,800 became poisoned and five died. Their symptoms were characteristic of acetylcholinesterase inhibition and the episodes of poisoning were epidemiologically associated with exposure to malathion, 50% water dispersible powders (wdp), some samples of which contained greater than normal amounts of isomalathion (Baker et al., 1978).

Since 1957 it has been known that other organophosphorus compounds can increase the toxicity of malathion (Frawley et al., 1957; Murphy and DuBois, 1957; Cohen and Murphy, 1970) by prevention of its detoxification normally brought about by its enzymic hydrolysis (Murphy and DuBois, 1957; Cook et al., 1958;

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Cohen and Murphy, 1971a). Evidence has been provided of impurities in malathion capable of inhibiting carboxylesterase in vivo (Murphy, 1967).

Prior to 1972 the oral toxicity of technical malathion for rats has been quoted as an LD_{50} of 1,000–2,000 mg/kg. However, two papers have indicated the effect of other substances on its toxicity. The first by Pellegrini and Santi (1972) implicated various trimethyl phosphorothioates; after their removal chemically, the oral toxicity of malathion to rats was reduced from 1,580 mg/kg to 8,000 mg/kg. A very recent paper quite independent of the outbreak of poisoning (Umetsu et al., 1977) indicated that isomalathion and other phosphates in technical malathion may contribute to its toxicity.

In this paper the toxicity of malathion samples taken in the field after various periods of storage has been compared with their content of various impurities. In addition, by the examination of mixtures with pure malathion, an assessment of the relative importance of various possible impurities has been made.

Methods

Analytical Methods. Samples of malathion wdp or technical were analysed for malathion content by the gas chromatographic method described by Wayne (1978) with the substitution of a column packed with 7 · 5% OV-210 on Chromosorb W HP in lieu of 5% SP-2401 on Gas Chrom. Q. Phosphorus and sulfur containing minor compounds with the exception of O,O,S-trimethyl phosphorothioate and O,S,S-trimethyl phosphorodithioate were determined by temperature programmed gas chromatography using the same column described for analysis of malathion. The above trimethyl esters were analyzed by temperature programmed gas chromatography using a 180 cm × 2 mm glass column packed with 7 · 5% OV-275 on Chromosorb W HP. Although many phosphorus and/or sulfur containing compounds were quantitated by the above procedures only those containing phosphorus are considered in this paper and are listed in Table 1.

Table 1. Structural formulae and chemical names of compounds

Name	Structural formula/chemical name
Malathion	$C_2H_5OC(O)CH[SP(S)(OCH_3)_2]CH_2C(O)OC_2H_5$ diethyl[(dimethoxyphosphinothioyl)thio]-butanedioate
Mixed ester	$CH_3OC(O)CH[SP(S)(OCH_3)_2]CH_2C(O)OC_2H_5$ ethyl methyl[(dimethoxylphosphinothioyl)thio]-butanedioate
Malaoxon	$C_2H_5OC(O)CH[SP(O)(OCH_3)_2]CH_2C(O)OC_2H_5$ diethyl[(dimethoxylphosphino)thio]-butanedioate
Isomalathion	$C_2H_5OC(O)CH[SP(O)(OCH_3)(SCH_3)]CH_2C(O)OC_2H_5$ diethyl[(methoxymethylmercaptophosphino)thio]-butanedioate
–	$(CH_3O)_2P(S)SP(S)(OCH_3)_2$ tetramethyl thiodiphosphate
–	$(CH_3O)_2P(S)SCH_3$ O,O,S-trimethyl phosphorodithioate
–	$(CH_3O)_2P(O)SCH_3$ O,O,S-trimethyl phosphorothioate
–	$(CH_3S)_2P(O)OCH_3$ O,S,S-trimethyl phosphorodithioate

Determination of Toxicity. Acute oral LD_{50} determinations were carried out using female Lac : P rats of 170—200 g unstarved weight and 9—10 weeks of age. All rats were housed in an air conditioned animal room at a temperature of 19—21° C and a relative humidity of 50—70%. Prior to dosing, rats were deprived of food for 15—18 h overnight but given free access to water. All rats were usually dosed between 10—30 and 13—00 h and immediately given access to food. Determination of LD_{50} was carried out by the method of Weil (1952) using 6 rats per group and usually a geometric relationship between doses with a factor of 1.26. Sometimes with compounds of high toxicity a smaller factor between doses (1.12—1.15), or those with low toxicity, a larger factor (1.585) was used.

Malathion wdp was administered as a suspension in water (maximum concentration 400 g wdp/litre); for technical malathion and mixtures of pure compounds glyceral formal was the solvent (Sanderson, 1959). The calculations of LD_{50} values and confidence limits were made by the method of Weil (1952) or by probit analysis. The concentration of malathion in the solvent was always in the range of 200—800 g/l depending on the approximate toxicity found by a preliminary experiment on 2 rats per dose.

Preparation of Pure Malathion by Recrystallisation. These details are reproduced by kind permission of Dr. M. Thomsen of Cheminova, Copenhagen, Denmark, who supplied the material used in this study. A mixture of approximately 100 ml ethanol and 100 ml malathion are cooled by standing 1—2 h at −15° C. A crystal of malathion was then added and the mixture allowed to stand for a further 20—30 h. Malathion (80—90% of the total) then crystallised as a coherent crystal mass: the crystallisation was repeated as required. After the final crystallisation the supernatant was poured off, the malathion melted and the ethanol removed in a rotary evaporator at 55° C for 2 h. By this means all impurities were removed except "mixed ester" (one methyl group replacing an ethyl group in the diethylmercaptosuccinyl moiety of malathion) and "ethyl malathion" (one ethoxy group replacing a methoxy group attached to phosphorus). By GLC analysis of the recrystallised malathion the only impurities found were small amounts of these compounds.

Synthesis of Isomalathion and Various Trimethyl Phosphorothioates. Isomalathion was prepared by the procedure described by Miles et al. (1978).

O,O,S-trimethyl phosphorodithioate was prepared by the reaction of equimolar quantities of potassium O,O-dimethyl phosphorodithioate with methyl p-toluenesulfonate in acetonitrile at room temperature. After removal of the resulting salt by filtration, the solvent was evaporated and the product vacuum distilled. BP 64° at 0.5 mm Hg; yield 49.5%. Purity by GLC and NMR was estimated to be 98%.

Preparation of O,S,S-Trimethyl Phosphorodithioate. 52 g of O,O-dimethyl phosphorodithioate and 5 g of O,O,S-trimethyl phosphorodithioate were dissolved in 260 ml of acetonitrile and refluxed for 20 h. The flask was chilled and crystals of the potassium salt of S,S-dimethyl phosphorodithioate were collected by vacuum filtration. Yield 90%. 37.9 g of the above salt were suspended in 400 ml of acetonitrile and brought to reflux whereupon 20 ml of dimethyl sulphate were added and the mixture refluxed for 6 h. 10 ml of water were added and the mixture refluxed for an additional 1 h to destroy the excess dimethyl sulphate. After removal of the precipitate by filtration, the acetonitrile was removed in a rotary evaporator. The product was dissolved in chloroform, washed with water and dried over sodium sulphate. The product was vacuum distilled. BP = 70° at 0.3 mm Hg. The purity determined by GLC was 98%. The structure was confirmed by NMR.

O,O,S-Trimethyl Phosphorothioate. This compound was synthesized by a two step process $(CH_3O)_2P(S)Cl$ was hydrolyzed in aqueous KOH to form the salt $(CH_3O)_2P(O)S^-K^+$[3]. The aqueous mother liquor was then extracted with toluene and the aqueous phase evaporated on a vacuum rotary evaporator. Ethyl acetate was added and the mixture was then filtered to remove the insoluble KOH and KCl. The filtrate was vacuum rotary evaporated to dryness, leaving the partially purified salt. Yield of the salt was 85%. The salt was dried and then methylated with methyl iodide in acetonitrile. The acetonitrile was vacuum rotary evaporated and the product purified by partitioning between ether and water. The combined ether layers were evaporated and the product vacuum distilled. Yield from the salt was 40% of the pure $(CH_3O)_2P(O)SCH_3$ boiling at 68° C at 0.75 mm Hg.

Results

Toxicity of Recrystallised Malathion. The sample of recrystallised malathion (purity 99.7%) supplied to us by Dr. M. Thomsen was examined and found to have an oral LD_{50} to rats of 10,700 (9,300–12,300) mg/kg. From a comparison with other values in the literature (Table 2), we conclude that the oral LD_{50} of pure malathion is approximately 10,000 mg/kg body weight and in the rest of this paper the toxicity of other specimens will be referred to this figure.

After administration of recrystallised malathion there was little evidence of poisoning for the first six hours; some fatalities occurred in the first 20 h, although most were between 20 and 40 h.

Symptoms of Poisoning by Isomalathion or Trimethyl Phosphorothioates or -Dithioates or Mixtures of Each with Malathion. The onset of poisoning was rapid when combinations of malathion and impurities were used. With malathion plus

Table 2. The toxicity of malathion and some trimethyl phosphorothioates. For chemical structures see Table 1

Oral toxicity to rats (LD_{50}, mg/kg body wt)	Time of death	References
Malathion		
8,000	[a]	Pellegrini and Santi (1972)
12,500	[b]	Umetsu et al. (1977)
9,500	—	Sample supplied by cyanamid and tested by Umetsu et al. (1977)
10,700 (9,300–12,300)	6–40 h	Sample supplied by Cheminova (this paper)
Isomalathion		
120	[b]	Umetsu et al. (1977)
89	—	Cyanamid report CMD 67–68 (1967)
113 (90–143)	8–50 min	This paper
O,O,S-trimethyl phosphorodithioate		
638 (536–760)	4–24 h	This paper
450	[a]	Pellegrini and Santi (1972)
660	[b]	Umetsu et al. (1977)
O,S,S-trimethyl phosphorodithioate		
26 (20–35)	3–6 days	This paper
96	[a]	Pellegrini and Santi (1972)
110	[b]	Umetsu et al. (1977)
O,O,S-trimethyl phosphorothioate		
60 (51–71)	3–4 days	This paper
47	[a]	Pellegrini and Santi (1972)
260	[b]	Umetsu et al. (1977)

[a] Animals observed for 7 days

[b] 24 h mortality; animals observed for 2 days

isomalathion fasciculations were observed within 15—30 min and fatalities occurred between 1.5—22 h. With the combination of malathion with trimethyl phosphorothioates or dithioates the onset of poisoning was also rapid with fasciculations beginning from 0.5—2 h after dosing and fatalities occurring between 2.5—48 h, with occasional animals dying between 55 and 69 h.

With very high doses of O,S,S-trimethyl phosphorodithioate alone (10 × LD_{50}) the onset of poisoning was within 30 min, fatalities occurring at 5 h. The symptoms were typical of those expected from inhibition of acetylcholinesterase — fasciculations, salivation, lachrymation, urinary incontinence, laboured respiration, and weakness. However, rats dosed at or near the LD_{50} showed a very slow onset of poisoning and fasciculations were slight. After 24 h the rats were weak and ill with staining around the eyes; weight loss and fatalities occurred between 3 and 6 days. The toxicity (LD_{50} 26 mg/kg) of O,S,S-trimethyl phosphorodithioate is unexpectedly high (Table 2).

After poisoning rats with high doses of O,O,S-trimethyl phosphorothioate (20 × LD_{50}) fasciculations, laboured respiration and weakness were observed at 60 min and fatalities occurred within 5 h. Animals dosed with approximately 3 × LD_{50} gradually deteriorated in condition and were found dead 3—4 days later (Table 2).

Poisoning with O,O,S-trimethyl phosphorodithioate was not typical of an organophosphorus compound since fasciculations, salivation, lachrymation and urinary incontinence were not seen. Within 15—30 min rats were semi-prostrate with untidy coats and at 90—120 min were completely prostrate with very slow respiration. After higher doses (1.5 × LD_{50}) there was no evidence of corneal or foot reflex at this time. After lower doses rats were lethargic and, when stimulated, moved with an unco-ordinated, unsteady gait. Fatalities occurred between 4 and 24 h with one rat surviving for 72 h. Recovery from their narcotic state was observed between 2—4 days. These symptoms resembled those observed previously with some other organophosphorus compounds (Vandekar, 1957) (Table 2).

Toxicity and Composition of Malathion (wdp). In our consideration of the differences in the toxicity of the large number of specimens of malathion (wdp) manufactured by different firms and used and stored in different countries, we have confined ourselves to phosphorus-containing impurities. Amongst those determined by the GLC method in this study are O,O,S-trimethyl phosphorodithioate, tetramethyl thiodiphosphate, mixed ester, malaoxon and isomalathion. Of these, visual inspection of the analytical results showed that malaoxon (0—0.17%, w. malaoxon/w. malathion) could not be the cause of the increased toxicity. Although the toxicity of mixed ester of malathion (cf. Table 1 for chemical structure) is not known, the published toxicity of compounds derived from dimethyl-, diethyl-, and di-n.propylthiosuccinate esters (Dauterman and Main, 1966) indicates that the presence of mixed ester cannot be associated with increased toxicity.

The relation between the ratio of the contents of the impurities O,O,S-trimethyl phosphorodithioate, tetramethyl thiodiphosphate and isomalathion to malathion and its oral toxicity to rats has been examined (Fig. 1). Although the scatter of results is large, it is clear that the only impurity with an acceptable relationship between its content and toxicity is isomalathion.

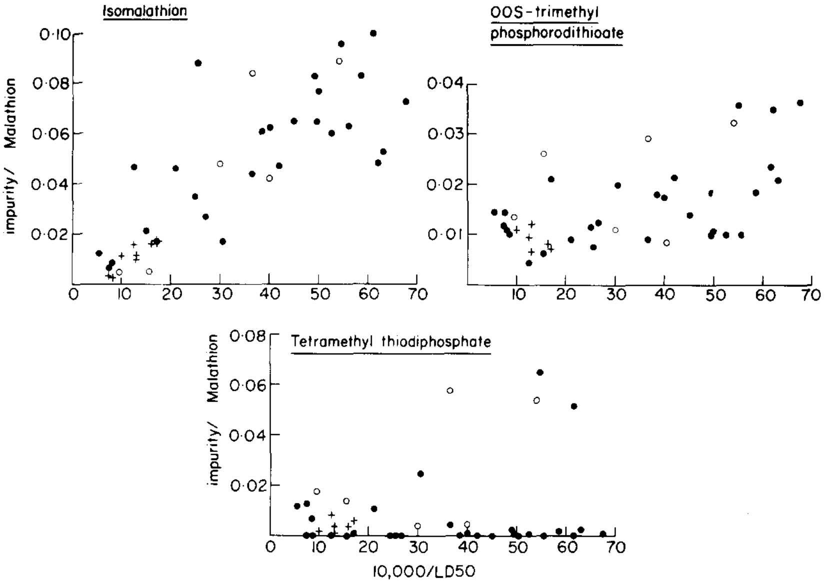

Fig. 1. Relationship between the oral toxicity to rats and the composition of various samples of malathion. (●) LD_{50} determined at Carshalton; (○) LD_{50} determined at Atlanta; (+) LD_{50} determined at Atlanta after laboratory storage at 38° C. Malathion technical 50% with Hi Sil and Malathion 50% wdp (both from Cyanamid, USA) were stored at 38° C and sampled at 0, 1, 2 and 3 months. The isomalathion content increased more in the 50% "in Hi Sil" sample than in the 50% wdp. On the vertical axis the ratio added compound/malathion has the units of wt/wt. On the horizontal axis in the ratio $10{,}000/LD_{50}$, the LD_{50} has the units mg/kg. Since the LD_{50} for purified malathion is approx. 10,000/kg (cf. Table 2) this ratio indicates the toxicity of the mixture relative to that of purified malathion

In order to test this hypothesis, the toxicity of mixtures of pure isomalathion with recrystallised malathion have been determined (Fig. 2). Increasing proportions of isomalathion increase the toxicity to an extent similar to, but not identical with, that shown in Fig. 1. For example, an isomalathion : malathion ratio of 0.04 increases toxicity 17-fold, whereas on average for a similar isomalathion : malathion ratio in wdp formulations a 30-fold higher toxicity over pure malathion is shown. Similarly, addition of pure isomalathion to technical malathion (isomalathion content 0.8% w/w) increases toxicity much more than in the case of analogous mixtures of pure isomalathion and recrystallised malathion (Fig. 2).

These results suggest that impurities other than isomalathion contribute to the toxicity of commercial malathion. In view of the presence of O,O,S-trimethyl phosphorodithioate and the work of Umetsu et al. (1977) the effects of various trimethyl phosphorothioates on the toxicity of purified malathion have been determined. The decreasing order of their activity in increasing the toxicity of malathion is O,S,S-trimethyl phosphorodithioate, isomalathion, O,O,S-trimethyl phosphorothioate and O,O,S-trimethyl phosphorodithioate (Fig. 2).

Potentiation of toxicity is defined as that toxicity of a mixture of substances which is more than the individual toxicities of its components assessed separately.

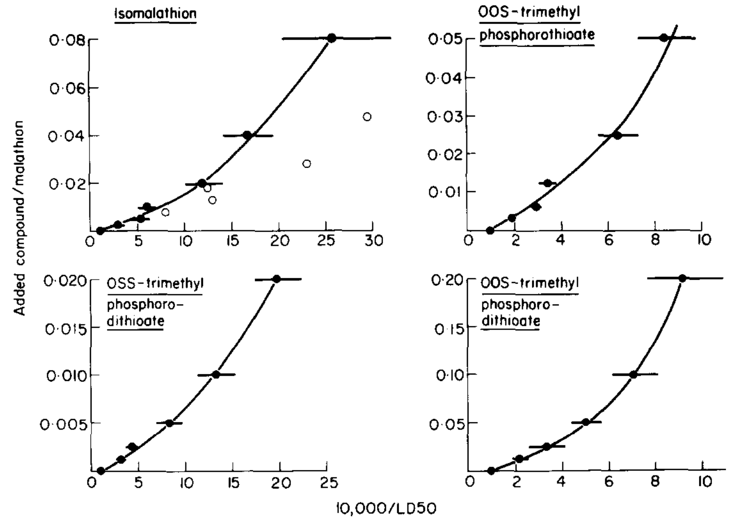

Fig. 2. Relationship between the oral toxicity to rats and the composition of mixtures of isomalathion, or trimethyl phosphorothioates with recrystallised malathion and isomalathion with technical malathion. (●) Mixtures with recrystallised malathion; (○) Mixtures of isomalathion with technical malathion. The first point plotted on the left relates the toxicity of technical malathion to its isomalathion content (0.8%). The derived relative activities of the added compounds in potentiating the toxicity of malathion are isomalathion – 1.0, O,S,S-trimethyl phosphorodithioate – 2.4, O,O,S-trimethyl phosphorothioate – 0.32 and O,O,S-trimethyl phosphorodithioate – 0.12. On the vertical axis the ratio impurity/malathion has the units of wt/wt. On the horizontal axis, in the ratio 10,000/LD_{50} the LD_{50} has the units mg/kg. Since the LD_{50} for purified malathion is approx. 10,000 mg/kg (cf. Table 2) this ratio indicates the toxicity of the mixture relative to that of purified malathion

Additive toxicity of two compounds (*A* and *B*) is given by

$$\frac{x}{a}+\frac{1-x}{b}=\frac{1}{c}$$

where a, b and c are the LD_{50} values of compounds A, B and the mixture respectively and x and $1-x$ are the proportional contents of A and B respectively in the mixture. When the toxicity of mixtures (col. 3, Table 3) is greater than the calculated additive value (col. 4, Table 3) potentiation is established. If these calculations are made for mixtures of isomalathion or trimethyl phosphorothioates with purified malathion potentiation is indicated for all compounds except O,O,S-trimethyl phosphorothioate (Table 3). However, the delayed time of death due to the trimethyl phosphorothioates imparts an unacceptable bias to these calculations. For example, the time of death after O,S,S-trimethyl phosphorodithioate and O,O,S-trimethyl phosphorothioate is spread over 3 to 6 days. In contrast, death after administration of malathion, to which either compound has been added, occurs mainly within one day. If the approximate LD_{50} required to cause death in one day is used (cf the bottom half of Table 3) and the recalculated additive toxicity (col. 4) compared with the measured LD_{50} (col. 3) obvious potentiation with all substances is indicated (Table 3).

Table 3. Influence of various organophosphorus compounds on the toxicity of malathion. For chemical structures see Table 1

Compound	Toxicity of compound (LD_{50}; mg/kg)[a]	Composition of mixture having stated LD_{50} (compound/malathion; w/w)[b]	Calculated additive toxicity of mixture (LD_{50}; mg/kg)[c]
Malathion	10,700	–	–
		measured LD_{50} = 1250 mg/kg	
O,S,S-trimethyl phosphorodithioate	26	0.0058	3,165
O,O,S-trimethyl phosphorothioate	60	0.043	1,241
O,O,S-trimethyl phosphorodithioate	638	0.135	3,421
Isomalathion	113	0.013	4,826
		measured LD_{50} = 500 mg/kg	
O,S,S-trimethyl phosphorodithioate	26	0.02	1,162
Isomalathion	113	0.053	1,794
		measured LD_{50} = 1250 mg/kg	
O,S,S-trimethyl phosphorodithioate	80–160[d]	0.0058	6,045–7,743
O,O,S-trimethyl phosphorothioate	400–800[d]	0.043	5,079–6,985
		measured LD_{50} = 500 mg/kg	
O,S,S-trimethyl phosphorodithioate	80–160[d]	0.02	2,928–4,750

[a] LD_{50} values taken from Table 2

[b] Composition of mixture having stated LD_{50} taken from Fig. 2

[c] For method of calculation see text

[d] Approximate LD_{50} 1 day after oral dosing

Discussion

From the evidence produced in this report and that previously published (Baker et al., 1978), there seems little doubt that isomalathion was the main determinant of the increased toxicity of malathion used for malaria control in Pakistan. Of the compounds determined by analysis a general correlation was shown only between increased toxicity and isomalathion content. This correlation applied to samples of malathion (wdp) manufactured by several firms from different countries and used in areas other than Pakistan. In retrospect, it is not possible to know with certainty the purity of the technical malathion before formulation; since laboratory storage at

38° C of malathion (wdp and with other diluents) increases both its isomalathion content and toxicity, it is possible that storage in a hot climate in Pakistan was the main cause of the increased isomalathion content of samples sprayed in the field (Fig. 1). From experiments with mixtures of recrystallised malathion and isomalathion it is clear that isomalathion potentiates the toxicity of malathion. The potentiation of malathion toxicity by other organophosphorus compounds has been known from animal experimentation since 1957 (Frawley et al., 1957; Murphy and DuBois, 1957) but the episode in Pakistan is its first demonstration in man.

In our present state of knowledge it is clear that commercial malathion contains other materials which can potentiate the toxicity of malathion (Umetsu et al., 1977; Table 3). Until recently malathion has been considered to have an oral LD_{50} for rats of 1,000—2,000 mg/kg but recent work indicates that the correct value is approximately 10,000 mg/kg (Table 1). The addition of isomalathion to a specimen of technical malathion does not produce the same relationship as that found for a mixture of isomalathion and recrystallised malathion (Fig. 2), indicating that other components in technical malathion contribute to the potentiation of its toxicity. Uncertainties about the nature of these other components indicate the need for further work. Possibilities include the various trimethyl phosphorothioates (Pellegrini and Santi, 1972; Umetsu et al., 1977; Fig. 2). Development of the GLC method of analysis has recently allowed the presence of O,S,S-trimethyl phosphorodithioate and O,O,S-trimethyl phosphorothioate to be determined in an early sample from Pakistan (J. W. Miles, unpublished).

The oral toxicity of recrystallised malathion to rats which is probably free from potentiating impurities is approx. 10,000 mg/kg. The proximal toxin, malaoxon has an oral toxicity of 158 mg/kg. (Dauterman and Main, 1966). The route of toxicity which would approximate to the production of malaoxon from malathion in the liver would be intravenous infusion. However, this value is not available. The oral toxicity of malaoxon to rats pretreated with tri 2-tolylphosphate to remove carboxylesterase (malathionase) is 23 mg/kg. (Dauterman and Main 1966). It seems reasonable to assume that the toxicity of malaoxon produced in the liver will be between these two values, 23 and 158 mg/kg. Such a difference in toxicity between malathion and malaoxon indicates that after oral administration of malathion only one molecule in 80—400 molecules arrive at the liver, a very high degree of protection by detoxification. The quantitative assessment of the contributions of various organs to the hydrolysis of malathion is not conclusively settled nor have the enzymes involved been characterised (Bhagwat and Ramachandran, 1975a and b).

Potentiation by isomalathion is in detail a rather complicated process (Fig. 3). After administration of pure malathion, as pointed out above, the predominant reaction (Dauterman and Main, 1966) is that with carboxyl esterase leading to inactive products (Main and Braid, 1962; Chen et al., 1969). Malaoxon is both an inhibitor of carboxyl esterases as well as a substrate (Dauterman and Main, 1966; Main and Dauterman, 1967). Kinetic studies have shown that detoxification is probably the dominant reaction (Main and Dauterman, 1967). Malaoxon is mainly produced in the liver and therefore detoxification will be mainly in this organ; after an oral dose of malaoxon detoxification can occur elsewhere; this may account for the big difference in toxicity of an oral dose to control and tri 2-tolylphosphate treated rats. Isomalathion is a direct inhibitor of esterases and by the inhibition of carboxylester-

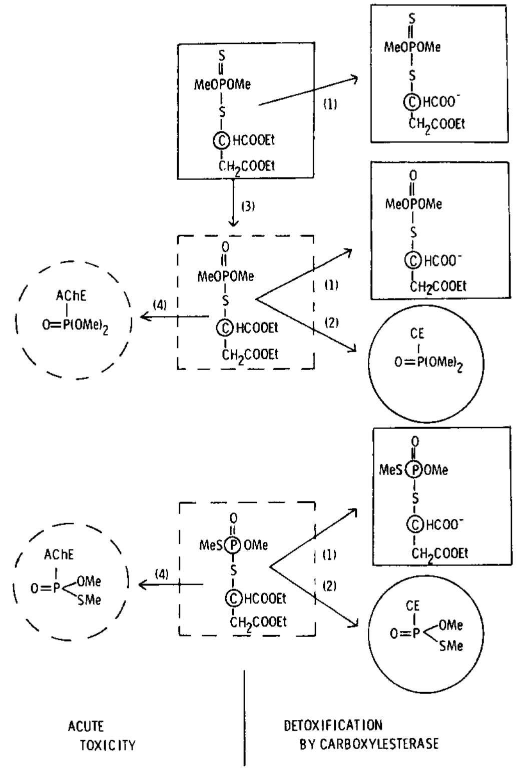

Fig.3 Reactions of malathion, malaoxon and isomalathion with carboxylesterase and acetylcholinesterase. Reaction (1) with carboxylesterase leads to inactive products; reaction (2) with carboxylesterases leads to its inhibition; reaction (3) is the conversion in vivo of malathion to malaoxon by the cytochrome P-450 drug processing system; reaction (4) with acetylcholinesterase results in acute toxicity. Structures in solid line squares are inactive as inhibitors in vitro; structures in dashed line squares are active inhibitors in vitro; structures in solid line circles are inhibited carboxylesterase (CE); structures in dashed line circles are inhibited acetylcholinesterase leading to acute toxicity. Atoms encircled with solid circles indicate asymmetric centres in the organophosphorus molecules

ase prevents the detoxification of malathion; very large numbers of other organophosphates have been shown to act in this way (Cohen and Murphy, 1971a and b; Casida, 1961; Casida et al., 1963). It is reasonable to assume that isomalathion, like malaoxon, will also be a substrate for carboxylesterase, though on present information the relative rates of detoxification and inhibitory activity cannot be predicted.

Isomalathion contains two asymmetric centres, one at the phosphorus atom and the other in the succinyl moiety of the leaving group. It is well known that the inhibitory power of organophosphorus compounds is much influenced by such stereochemistry (Aaron et al., 1958; Hilgetag and Lehmann, 1959; Keijer and Wolring, 1969; Ooms and Boter, 1965; Benschop, 1975; Wustner and Fukuto, 1974; Schoene, 1971, Ooms and Van Dijk, 1966; De Jong and Van Dijk, 1972; Boter and Van Dijk, 1969; Boter and Ooms, 1967). The enantiomers of diethyl malathion with an asymmetric carbon in the succinyl moiety are hydrolysed at different rates by rat liver carboxylesterase and those of diethyl malaoxon react at different rates with both acetylcholinesterase and rat liver carboxylesterase (Hassan and Dauterman, 1968). Therefore it seems likely that the reaction rates of the four possible enantiomers of isomalathion with both acetylcholinesterase and carboxylesterase will differ. The rates of hydrolysis of the ethyl succinyl group will certainly be influenced by the asymmetric carbon. Whether the asymmetric phosphorus atom will affect this reaction cannot be predicted.

Both isomalathion and O,S,S-trimethyl phosphorodithioate are in vitro inhibitors of rat liver carboxylesterase(s) (Aldridge, unpublished experiments). The very high activity of the latter in the potentiation of malathion toxicity was unexpected and is still not understood. The toxicology of the trimethyl phosphorothioates is unusual for deaths to occur between 3 and 6 days. Experiments are in progress to establish the cause of death but whatever this is, significant concentrations would be undesirable in insecticides to which spraymen are exposed daily.

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